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THE GROWTH OF THE VIRUS OF EPIDEMIC DIARRHOEA OF INFANT MICE (EDIM) IN ORGAN CULTURES OF INTESTINAL EPITHELIUM

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SUMMARY.—The virus of epidemic diarrhoea of infant mice (EDIM), never previously cultivated *in vitro*, has been grown in organ cultures of mouse ileum, caecum and colon. Virus was passed twice in cultures of caecum and 3 times in ileum, but failed to grow in mouse embryo fibroblasts.

THE virus of epidemic diarrhoea of infant mice (EDIM) has been recognized for some years. It has been transmitted by inoculating extracts of intestine or intestinal contents orally into baby mice which develop diarrhoea after about 1 week but usually recover (Kraft, 1957). The virus causes acute degenerative changes in the intestinal epithelium and virus particles have been seen in the cytoplasm of these cells (Adams and Kraft, 1967; Banfield, Kasnic and Blackwell, 1968).

We are trying to find conditions in which viruses which naturally inhabit the alimentary tract will multiply in cultures made from intestinal mucosa and it has already been shown that certain viruses can be grown in organ cultures of ileum, caecum, colon, and oesophagus whether they grow in tissue culture easily (Rubenstein and Tyrrell, 1970; Stenhouse, 1970; Dolin, Blacklow, Malmgren and Channock, 1970) or with difficulty (Rubenstein, Tyrrell, Derbyshire and Collins, 1970).

We have used the same techniques to determine whether organ cultures of the alimentary tract of the mouse support the growth of EDIM virus, which will not apparently grow in tissue culture and multiplies only in the alimentary tract *in vivo*.

MATERIALS AND METHODS

EDIM was a strain recovered by Dr. L. M. Kraft and kindly supplied by Dr. J. C. Parker of Microbiological Associates. One drop was given orally to 1–3 day old suckling mice. Most of an inoculated litter were evidently underweight and unhealthy by the 7th day after inoculation; only those animals in which definite diarrhoea could be detected by external inspection were classed as infected in the ratios presented later in this paper.

The intestinal tract and its contents were collected from animals at the height of the disease and either frozen at −70° or processed immediately. The tissue was disintegrated in approximately 10 volumes of bacteriological broth containing 2 per cent bovine plasma albumin and 1000 u. of penicillin and 1000 μg. of streptomycin per ml. The suspension was clarified and centrifuged at 3000 r.p.m. for 15 min. after which it was sterile on bacteriological culture. Small samples of each virus pool were stored at −70°. Mice were held in an isolation room but no attempt was made to isolate each box strictly from each other. However, by 1 week after inoculation the litters were clearly normal or infected and titration experiments were terminated at this stage to avoid the confusing effects of cross infection. Infectious titres in suckling mice were calculated using the method of Karber.

The basic experiment consisted of the inoculation of each of a group of organ cultures, prepared as previously described (Rubenstein and Tyrrell, 1970), with 0·2 ml. of virus suspension containing $10^{2.45}$ TCD_{50} infectious units. The cultures were treated as in the previous experiments but in these 0·2 per cent "HEPES" buffer (N-2-hydroxyethylpiperazine N'-ethanesulphonic acid), previously shown to support the maintenance of cell cultures and pituitary tissue in organ culture (Shipman, 1969; Fisk and Pathak, 1969), was added to the culture medium. Specimens were fixed for electronmicroscopy by the method of Hirsch and Fedorko (1968), embedded in Epon and stained with uranyl acetate and lead citrate.

RESULTS

The experimental results are shown in Table I. No virus was recovered from cultures 3 hr after inoculation but it appeared later in cultures of ileum, caecum and colon, but not in oesophagus or duodenum.

TABLE I.—*Recovery of Virus from Organ Cultures Inoculated with EDIM*

Tissue	Day of harvest		
	0	1	2–8
Oesophagus	—	Neg	Neg
Duodenum	—	Neg	Neg
Ileum (Expt. 1)	Neg	Neg	4/7
Caecum (Expt. 1)	Neg	1/6	4/11
(Expt. 2)	Neg	3/10	3/10
Colon	Neg	Neg	2/7

An attempt was also made to pass the virus in cultures of ileum. These were inoculated with a pool of fluids harvested on the 2nd to 8th day of the previous experiment. The results are shown in Table II. No virus was detected until 3 days after inoculation.

TABLE II.—*Passage Experiments*

Tissue	Virus inoculum	Days after inoculation						
		0	1	2	3	4	5	6
Ileum (Expt. 2)	2–8 day pool of ileum expt. 1	0/7	0/7	0/7	4/6	—	4/7	0/6
Ileum (Expt. 3)	Day 5 pool of ileum expt. 2	—	—	—	—	—	4/7	—
Caecum (Expt. 3)	Day 5 pool of caecum expt. 2	—	—	—	—	—	6/11	—

(Denominator denotes number of mice inoculated. Numerator denotes number of mice infected.)

The harvests taken at day 5 of the original experiment with caecal tissue (Experiment 1) and at day 5 of the 2nd experiment with ileum (Experiment 2) were inoculated onto further cultures of the same tissue type. They were harvested as before, and the specimens collected 5 days after inoculation produced diarrhoea in suckling mice (Table II) to a titre of $10^{2.38}$ TCD_{50}. Organ cultures of caecum were fixed for histology after inoculation with infectious harvests. Sections show progressive flattening of the villi. The mucosa eventually loses its single columnar pattern, becomes pseudostratified, and finally only islands of epithelial cells remain (Fig. 1). No difference was observed between infected and uninfected cultures, although inclusion bodies have been observed in the mucosal epithelial cells of the intestine of infected mice (Pappenheimer and Enders, 1947). Figure 2 shows the electronmicroscopic appearance of a thin section of a culture

fixed at 3 days and demonstrates that the ultrastructure of some cells is well preserved and it is presumably these which are susceptible to virus infection.

Electronmicroscopic examination of infected cultures failed to reveal virus particles of EDIM virus, although these were seen in sections of the intestinal epithelium of mice infected *in vivo*. Both infected and uninfected organ cultures from the germ-free mice revealed virus-like particles approximately 85 nm in diameter. Similar particles were also visualized by negative staining of pellets of organ culture fluid (Fig. 3).

Harvests taken from the cultures of ileum and caecum after the later passages and known to be infectious for suckling mice were inoculated onto primary and secondary embryo mouse fibroblasts: no cytopathic effect was observed and media collected 2–5 days after inoculation failed to produce diarrhoea in suckling mice.

DISCUSSION

There has been no previous report of the growth of EDIM virus *in vitro*. The negative results in cultures of oesophagus and duodenum make it unlikely that the persistence of the virus in cultures of other tissues is due to non-specific protection of the infectivity of the initial inoculum by the presence of tissue. Also, the absence of virus from 0 to 2 days and its subsequent appearance and disappearance strongly suggest that it had multiplied during serial passage in the cultures—the estimated final dilution of the original inoculum at the end of the 3rd passage was 10^{-21}

The rapid degeneration of the mucosal cells of the epithelium suggests that the virus might be growing in cells other than those of the mucosa. However, attempts to grow the virus in embryo mouse fibroblasts were not successful. This indicates that the virus grows only in " organised " or differentiated intestinal tissue.

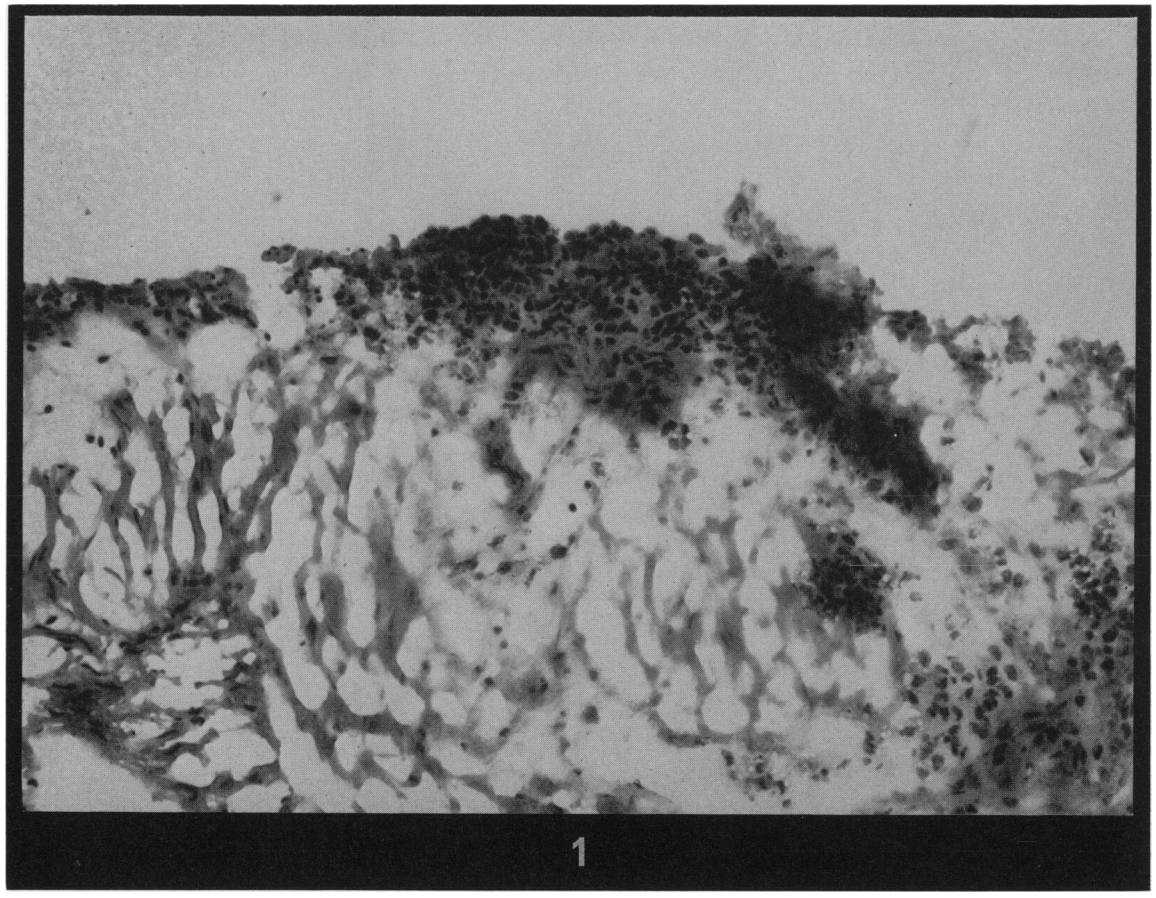
Inclusion bodies have previously been detected in the mucosal epithelial cells of the intestines of mice infected with EDIM (Pappenheimer and Enders, 1947), but were not seen in sections of the intestine after *in vitro* culture. By immunofluorescence the presence and site of respiratory viruses have been detected in organ cultures of trachea (Hers, Kuip and Masurel, 1968). The same technique has shown the presence of Sendai virus in cultures of human embryo ileum (Rubenstein and Zisman, unpublished). but was unsuccessful for EDIM in mouse intestinal cultures in this study. EDIM virus particles have not yet been identified in infected organ cultures of mouse intestine, which is not surprising in view of the low virus titres obtained from them. Nonetheless, the identification of virus-like particles in these organ cultures indicates that electronmicroscopy offers a direct method of virus identification in *in vitro* preparations of organized intestinal tissue.

EXPLANATION OF PLATES

FIG. 1.—Organ culture of mouse caecum 4 days after preparation and inoculation with EDIM. A typical island of dedifferentiated cells. Identical appearances were seen in uninfected cultures. Fixed formol-saline, stained haematoxylin and eosin. ×130.

FIG. 2.—Section of mucosal epithelium 3 days after preparation, showing parts of several cells, one of which appears normal and the remainder show varying degrees of degeneration. ×22,000.

FIG. 3.—(a) Virus-like particles seen in sections of infected and uninfected cultures of germ-free mouse ileum. (b) A cluster of virus-like particles seen in negatively stained high speed pellets of medium from uninoculated cultures. ×150,000.



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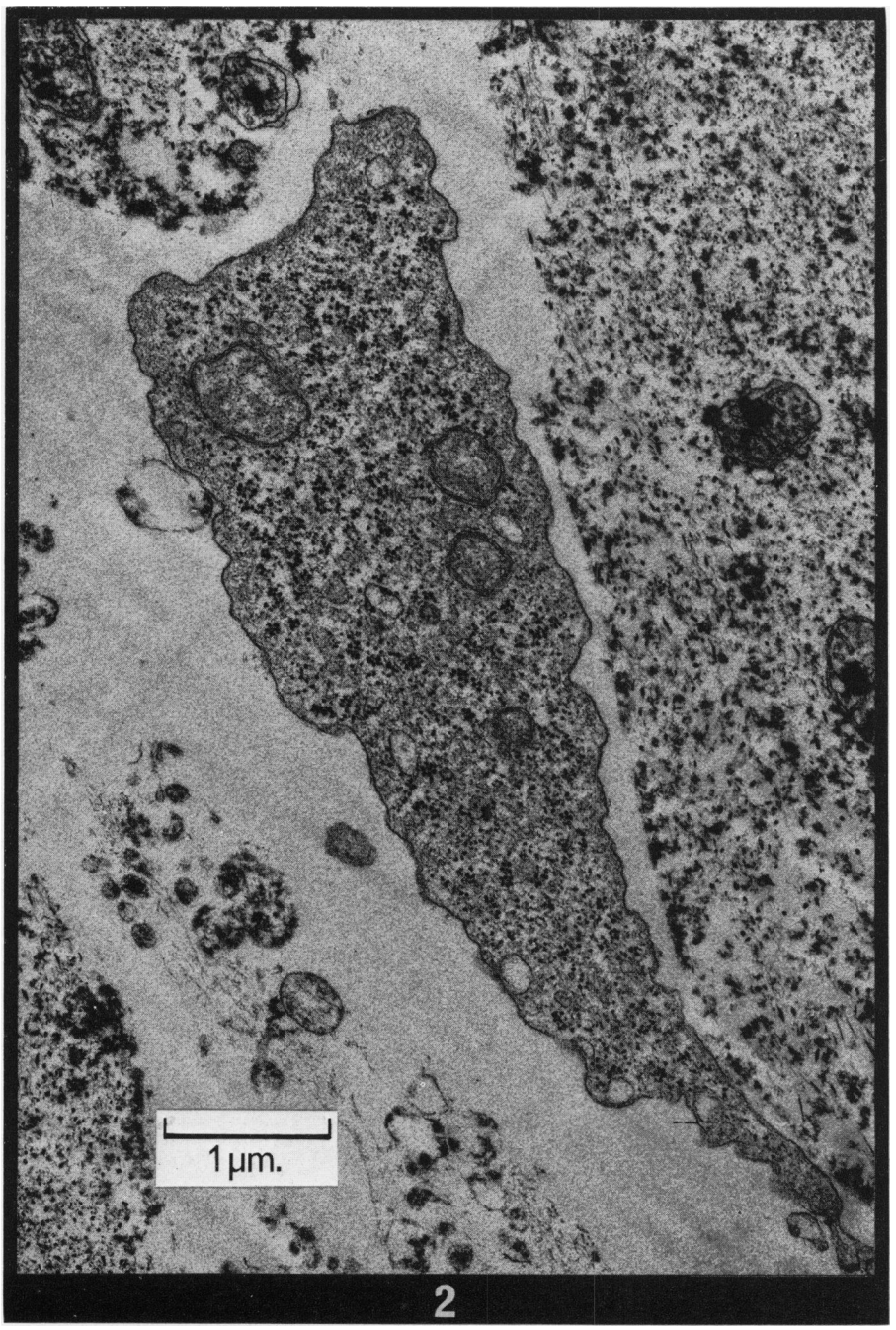

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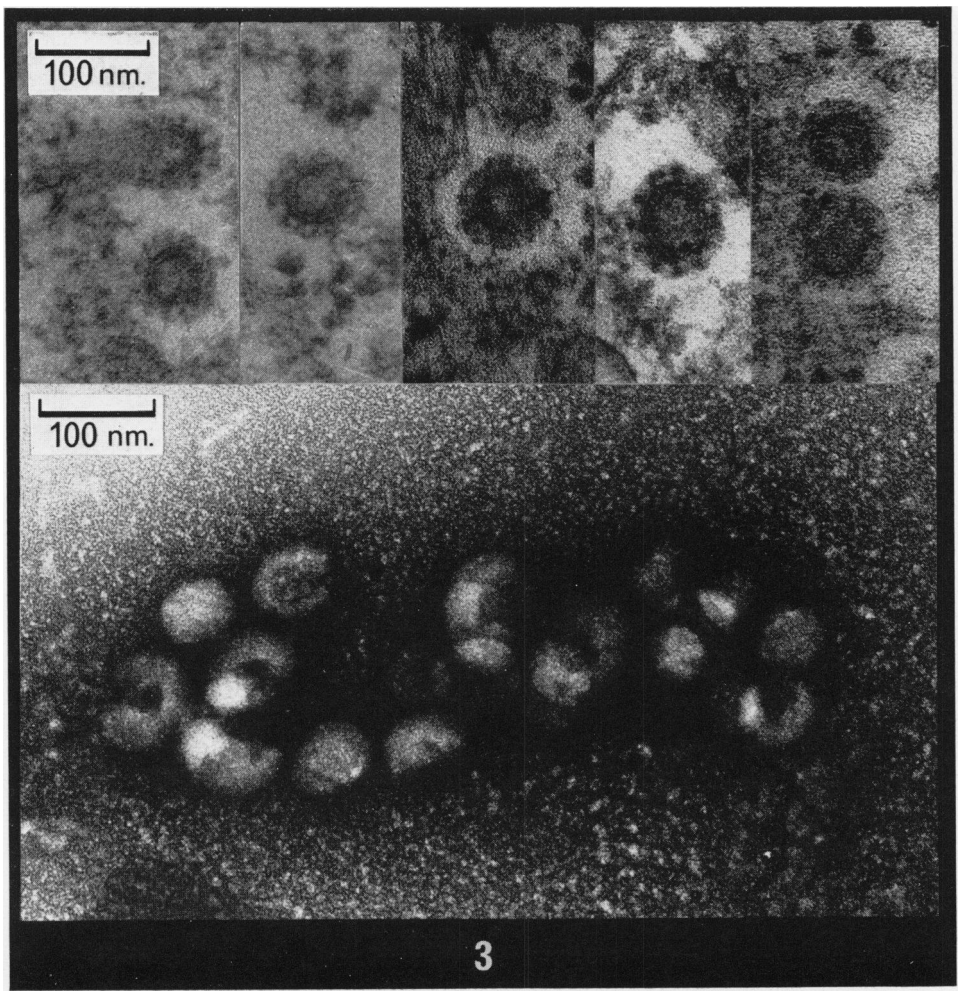

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The successful growth in intestinal organ cultures of animal viruses (Rubenstein *et al.*, 1970) which produce gastroenteritis indicates that the system may be of value in attempting to propagate and identify the aetiological agent (or agents) responsible for acute non-bacterial gastroenteritis of man which has been shown to be transmissible and to pass a bacteria-holding filter but has never been cultivated (Reimann, Hodges and Price, 1945; Gordon, Ingraham and Korns, 1947; Jordan, Gordon and Dorrance, 1953).

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